Life Technologies™
Your Molecular & Cell Technology Partner

GeneProof
Molecular diagnostics for your routine

Package insert

GeneProof Human Papillomavirus (HPV) PCR Kit

CE IVD

In vitro diagnostic medical device

The kit has been manufactured according to EC Directive 98/79/EC as an *in vitro* diagnostic medical device and it has been designed for professional use in specialized clinical and research laboratories.

KIT CONTENT

ISEX Version
IS detected from sample
Nucleic acid isolation and PCR inhibition control

REF	HPV/ISEX/025 25 rxn	HPV/ISEX/050 50 rxn
MasterMix HPV16	1x750 µl	2x750 µl
MasterMix HPV18	1x750 µl	2x750 µl
MasterMix HPV53	1x750 µl	2x750 µl
MasterMix HPV26/34	1x750 µl	2x750 µl
Positive Control HPV	3x200 µl	6x200 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kits should be transported and stored at temperatures between -85 °C and -10 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept. Repeated freezing and thawing of the kit components may result in lower detection quality.

TECHNICAL SPECIFICATION

Target sequence	*E2/E4* gene
Specificity	Human papillomavirus with optional identification of high-risk subtypes
	Master mix 16 group 9 - detected types: 16, 31, 33, 35, 52, 58, 67
	Master mix 18 group 7 - detected types: 18, 45, 97, 59, 39, 68, 70
	Master mix 53 group 6 - detected types: 30, 53, 56, 66
	Master mix 26/34 group 5 and 11- detected types: 26, 51, 69, 82, 34, 73
Clinical sensitivity (LOD)	for HPV 16 reaches 500 IU/ml (500 GEq/ml)
	for HPV 18 reaches 400 IU/ml (400 GEq/ml)
Sample types	Cervical, urethral, vaginal or penile swab
Quality control	regularly tested by QCMD and Instand e.V. External Quality Assessment Panel

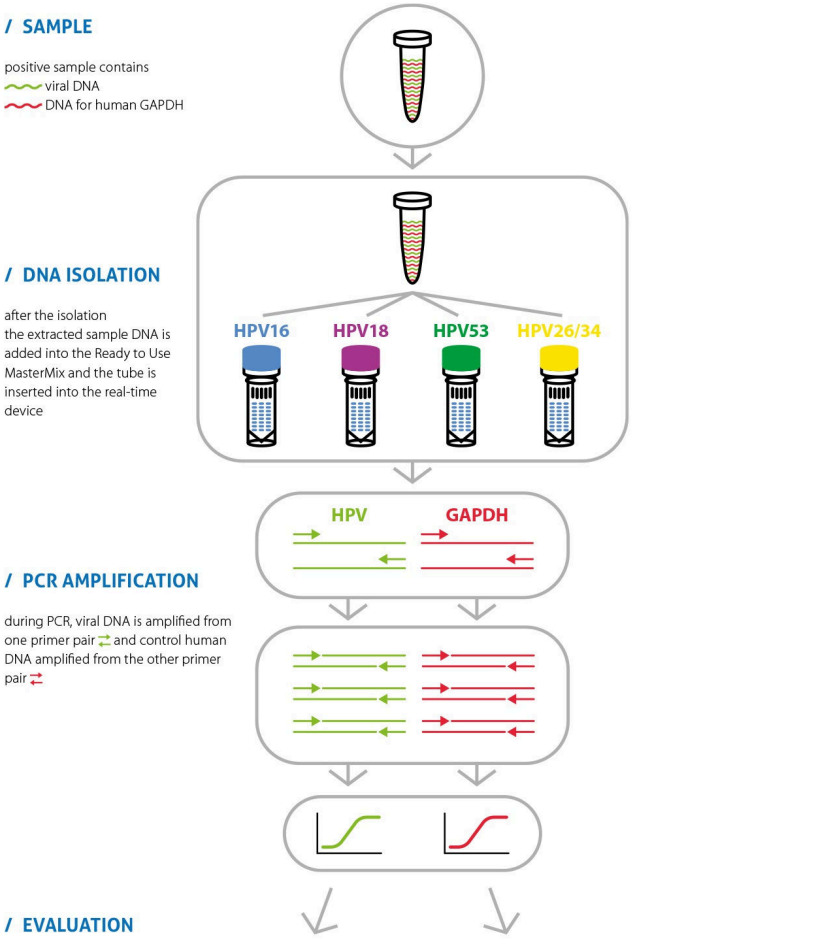
METHOD PRINCIPLES

The PCR kit is designed for the detection of high-risk types of Human Papillomavirus (HPV) by the real-time Polymerase Chain Reaction (PCR) method. The HPV detection is based on the amplification of a specific conservative DNA sequence in the area of genes *E2*/*E4* and measuring the amplification product concentration using PCR process and fluorophore labelled probes. HPV presence is indicated by the FAM and Cy5 fluorophore fluorescence growth. For the DNA isolation quality control and possible PCR inhibition control there are primers and probe for *GAPDH* gene amplification present in the reaction mix. Amplification of *GAPDH* gene is indicated in the HEX fluorophore fluorescence channel. The detection kit utilizes the "hot start" technology, minimizing non-specific reactions and assuring maximum sensitivity. Ready to Use MasterMix contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by amplification products. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

ISEX version

Internal Standard is detected from the sample. This PCR kit version enables both PCR inhibition control and nucleic acid purificaion process efficiency control.

MICROBIOLOGICAL DNA DIAGNOSTIC TECHNOLOGY



USER MANUAL

SAMPLING AND SAMPLE STORAGE

It is possible to use cervical, urethral, vaginal or penile swab for HPV detection. Using self-testing devices (e.g. Evalyn Brush) is also possible. Swab specimen can be stored up to 48 h in temperature range from +2 °C to +8 °C. For long term storage it's necessary to keep samples frozen at -85 °C to -10 °C.

NUCLEIC ACID PURIFICATION

Nucleic acid isolation should be performed by isolation kits available at the market according to protocols for the particular clinical material isolation. The manufacturer recommends the following isolation kits:

GeneProof PathogenFree DNA Isolation Kit
croBEE NA16 Nucleic Acid Extraction System

PCR SETUP

1. Add 30 µl of MasterMix into PCR tubes.

2. Add 10 µl of the isolated nucleic acid sample or 10 µl of Positive Control into the individual PCR tubes. The final reaction mix volume will be 40 µl.
It is necessary to keep all components at +2°C to +8°C during the PCR preparation.

3. Close the tubes, centrifuge shortly, insert them into the device and let them amplify according to the following PCR profile.
Be very careful when handling the Positive Control or the clinical material, incorrect handling could result in contamination and the consequent impairment of the kit components or the MasterMix! The manufacturer is not responsible for the kit impairment due to incorrect handling.

AMPLIFICATION PROFILE

Step	Temperature	Time	Data collection	Cycles
1. Hold	37 °C	2 min		1
2. Hold	95 °C	10 min		1
3. PCR	95 °C	5 s	FAM+HEX+Cy5	45
	60 °C	40 s		
	72 °C	20 s		

VALIDATED INSTRUMENTS

GeneProof PCR kits are designed for use with real-time devices from various manufacturers. This PCR kit has been validated with the following devices:

CFX96™/Dx Real-Time PCR Detection System
Applied Biosystems 7500 Real-Time PCR System
LineGene 9600*

* Validation applies to a device model providing detection in the following channels: FAM, HEX and Cy5.
GeneProof diagnostic kits are continually validated with various types of devices. Please request the current list at support@geneproof.com.